Leinco Technologies, Inc.
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Anti-Human CD18/CR3

Alexa Fluor® 647

Hybridoma Monoclonal Antibody

Product Information

Product No.:	C577AF
Clone:	KIM127
Isotype:	Mouse IgG1 κ

Product Description

Specificity:
Anti-CD18/CR3 antibody (clone KIM127) recognizes an epitope on the leukocytic cell-adhesion molecule (Leu-CAM) common β chain, CD18. As such, this antigen is considered part of the Leu-CAM β integrin family of adhesion molecules. Activity is directed against immunoprecipitated human CR3 and lymphocytes expressing LFA-1. KIM127 binds to pre-activated integrins.

Antigen Distribution:
Members of the Leu-CAM β integrin family of adhesion molecules are widely distributed on circulating leukocytes.

Background:
Integrins are a large family of *α/β* heterodimeric transmembrane molecules that mediate adhesion, migration, cell survival, and cell differentiation[1]. The leukocytic cell-adhesion molecule (Leu-CAM) family has three members, LFA-1 (CD18/CD11a), CR3 (CD18/CD11b), and p150,95 (CD18/CD11c), all of which share the β chain CD18[2].

KIM127 recognizes a temperature sensitive epitope on CD18, binding to lymphocytes that express LFA-1 as well as to immunopurified CR3[3]. KIM127 promotes LFA-1 and CR3-dependent adhesion events and enhances aggregation of the human B lymphoblastoid line JY and human T cell line MOLT-4. This aggregation is completely blocked by anti-LFA-1 antibody DA36. KIM127 is not associated with increased surface expression of CR3 or LFA-1.

KIM127 recognizes residues Gly 504, Leu 506, and Tyr 508 of CD18 in a cysteine-rich repeat located in the long loop protruding from the C-terminal end of the integrin-epidermal growth factor-like (I-EGF) 2 domain[4,5,6]. The epitope is near the extreme bend (genu) between the integrin headpiece and stalk, buried deep in the crevice formed by the bend between the α subunit thigh and calf-1 domains, and is only accessible when the integrin is extended in its activated conformation[6]. In resting integrins, the KIM127 epitope is shielded by the α subunit[5]. KIM127 preferentially reacts with activated β_2 integrins and free β_2 subunit compared with resting β_2 integrins. A switchblade-like action that extends the headpiece of the integrin away from the plasma membrane is thought to expose the KIM127 epitope when activated[6].

Activation of LFA-1 ligand binding by Mn^{2+}, and to a lesser degree by Mg^{2+} in the absence of Ca^{2+}, is positively correlated with increased expression of the KIM127 epitope[5]. PMA also induces a small but consistent increase in KIM127 expression.

KIM127 was obtained from a BALB/c mouse immunized with CR3 immunopurified from a human white cell lysate[3]. Hybridoma lines were created by fusing spleen cells with Sp2/0 cells and screening by ELISA for their ability to bind to immunopurified CR3 and then for their ability to promote aggregation of different cell types. Mildly denaturing conditions are required during Western blotting because boiling the sample destroys the KIM127 epitope.

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Known Reactivity Species:
Human

Format:
Alexa Fluor® 647

Formulation
This Alexa Fluor® 647 conjugate is formulated in 0.01 M phosphate buffered saline (150 mM NaCl) PBS pH 7.4, 1% BSA and 0.09% sodium azide as a preservative.
Alexa Fluor® is a trademark of Life Technologies Corporation.

Storage and Stability
This Alexa Fluor® 647 conjugate is stable when stored at 2-8°C. **Do not freeze.**

Applications
Applications and Recommended Usage (Quality Tested By Leinco):
WB[3],
FC[3,5,7,8,9,10,11,12]

Other Applications Reported in Literature:
ELISA[3],
IP[13],
IF Microscopy[12, 14, 15, 16]

Country of Origin
USA

References
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